Laboratory Animal Science

Hematologic Characterization of Naturally Occurring Malaria *(Plasmodium inui)* in Cynomolgus Monkeys *(Macaca fascicularis)*[1,2,3]

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Summary | Twenty of 47 recently imported cynomolgus monkeys *(Macaca fascicularis)* were found to have malarial infections. The agent identified was *Plasmodium inui*. All infections were subclinical in nature. Parasitemias ranged from 10 to 900 parasites/mm^3 of whole blood. Pre- and post-treatment hematologic values were evaluated following treatment with chloroquine. Treatment was effective in clearing parasitemias from 13 of 14 infected monkeys. Pretreatment values of hematocrit, hemoglobin, and mean corpuscular volume were significantly different in infected animals compared to noninfected animals. While post-treatment hemoglobin and hematocrit values returned to noninfected control levels, mean corpuscular volume values of infected animals remained significantly lower in the post-treatment period.

Key Words | Malaria — *Plasmodium* — *Macaca*

Malaria is a naturally occurring infectious disease of mammals, birds, and reptiles which manifests itself as a syndrome with various degrees of anemia, fever, malaise, and splenomegaly. Infection is caused by blood-borne protozoan parasites of the genus *Plasmodium*. The parasite is transmitted by infected mosquitoes. Following a stage of exoerythrocytic schizogony in the liver of the mammalian host, merozoites are released into the bloodstream and subsequently parasitize circulating erythrocytes. In the erythrocyte, an asexual reproductive cycle is established, with hemoglobin being utilized by the developing parasites. Sexual forms, gametocytes, also develop within erythrocytes allowing continuation of the life cycle within the arthropod vector. Infected erythrocytes are ruptured by the developing intracellular parasites or removed from circulation by the reticuloendothelial system.

Various species of *Plasmodium*, including *P inui, P cynomolgi, P knowlesi, P coatneyi*, and *P fieldi* are known to infect cynomolgus monkeys, and mixed infections are common (1,2). The incidence of infection is dependent on vector availability and host-parasite antigenic relationships (1,3,4). While an abundance of information is available with reference to nonhuman primate malarias, there is a lack of published data evaluating the effects of naturally occurring malaria infections on the hematologic values of the cynomolgus host.

Impetus for this investigation was provided by: (a) the importance to the research community of critically defining intercurrent disease processes in experimental animals; (b) the growing role of the cynomolgus monkey in biomedical research; and (c) the widespread use of hematologic values as indicators of physiologic response in a variety of research endeavors. The purpose of this investigation was: (a) to determine the incidence, parasite counts, and etiologic agent of malarial infections in cynomolgus monkeys recently imported to the United States; (b) to characterize the effects of the infections on the hematologic values of this primate species; and (c) to evaluate treatment with chloroquine.

Materials and Methods

Animals: Forty-eight, adult, male *Macaca fascicularis* were obtained from a commercial importer[4] following a minimum 45-day conditioning period. The countries of origin were the Philippines and Indonesia. One monkey died within hours of arrival at our quarantine facility of an undiagnosed illness. The remaining 47 animals were found to be clinically normal by physical examination and daily observation for 2 weeks. Animals were housed indoors in aluminum squeeze cages, provided a commercial monkey diet[5] twice daily with fruit supplementation twice weekly. Water was provided *ad libitum* via an automatic watering system. Room environment was maintained at 24 ± 1°C and 40 ± 10% relative humidity with a 12-hour light-dark cycle.

Experimental design: The investigation was scheduled for a 6-week period beginning 2 weeks after the animals' arrival at our quarantine facility. During the first week of study, blood samples were collected on three consecutive days to determine incidence, parasite identity,

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[4]Worldwide Primates, Miami, FL.
[5]High Protein Monkey Chow. Ralston Purina Co, St Louis, MO.

and parasite counts. With this initial information, 14 infected and 14 noninfected animals were randomly selected for study. Blood samples were taken for hematologic determinations and parasite counts at 1 week intervals throughout the study period. Hematologic determinations included total erythrocyte count, hemoglobin, hematocrit, mean corpuscular volume, and total and differential leukocyte counts. Reticulocyte counts were not performed, as previous experience at this institute had failed to demonstrate a reticulocyte response at the levels of parasitemias encountered. All infected animals received 5 mg/kg chloroquine base[6] intramuscularly once daily for 7 days between the samplings in the third and fourth weeks. Nine of the other noninfected animals also were treated with this regime.

Technical procedures: All blood collection was performed using ketamine hydrochloride,[7] intramuscularly, 5 mg/kg, for restraint. Blood samples for parasite counts and morphologic identification were obtained by inserting a 25-gauge needle into the saphenous vein and collecting from the hub of the needle using heparinized microhematocrit capillary tubes. Thick and thin Giemsa-stained blood smears were used to identify the species of malaria and establish parasite counts. Determination of parasite identity was based on morphologic characterization on thin smears. Blood samples for complete blood counts were collected with a 20-gauge needle from the femoral vein into 2-ml vacuum tubes[8] containing EDTA as an anticoagulant. Hematologic determinations were performed with the use of an automated blood cell counter and hemoglobinometer.[9]

Statistical analysis: Statistical analysis was performed using the analysis of variance (5). Effects of treatment on infected monkeys were measured and analyzed. The effects of infection between malaria-positive monkeys and noninfected monkeys also were compared. Treatment effects were analyzed by comparing the mean of three pre-treatment values to each of three post-treatment values using a completely randomized design. Effects of infection were analyzed separately for the pre- and post-treatment periods utilizing a completely randomized design with two factorials. Differences between treated and untreated noninfected monkeys also were measured. Significance was defined at $p \leq 0.05$.

Results

The etiologic agent of patent infections was in all cases *P inui*. Twenty of the 47 monkeys were infected. Single-day determinations resulted in incidence rates of 31, 28, or 21%, while accumulated data from the three consecutive days gave the reported rate of 43%. All infections were subclinical in nature with parasitemias ranging from 10 to 900 parasites/mm^3 of whole blood. Treatment was successful in clearing parasitemias in 13 of 14 monkeys, with no observable adverse side-effects. The one nonresponder and six infected monkeys not included in the study were allowed to remain infected for further clinical studies.

Hematocrit, hemoglobin, mean corpuscular volume, and erythrocyte values were lower in infected than in noninfected monkeys before treatment (Figure 1). These differences were significant ($p<0.05$) in all except the erythrocyte counts (Table 1). The hematocrit, hemoglobin, and erythrocyte counts of infected monkeys showed a significant increase following treatment, to where there were no longer significant differences between them and noninfected monkeys. The mean corpuscular volume of infected animals remained at the same level for the 3-week post-treatment period observed, which was still significantly lower than the noninfected monkeys. No significant differences were found between treated and untreated noninfected monkeys throughout the study period; the noninfected monkeys thus were considered a homogeneous group for subsequent analyses. Although total and differential leukocyte counts also were measured, within group variability was great, and no significant differences were observed between any of the groups before or after treatment.

Discussion

This investigation was conducted in order to characterize in part the effects of naturally occurring malaria in cynomolgus monkeys by observing alterations of hematologic values in infected animals. The data presented in Figure 1 show that cynomolgus monkeys may have significant hematologic alterations associated with low grade malarial infections. Table 1 presents the significance of the findings. Although these clinically inapparent infections did not uniformly cause fluctuations of hematologic values outside the published normal range (6), the alterations are significantly different from noninfected control values and clearly compromised the animals' utility in certain types of research.

The changes in erythrocyte count, hematocrit, and hemoglobin are relatively straightforward in malarial infections and are associated with the destruction of erythrocytes by developing intracellular parasites or removal of altered erythrocytes by the reticuloendothelial system (6). An explanation for the reduction of mean corpuscular volume of infected animals is more difficult. Microcytic anemias are generally caused by the effects of chronic infectious disease or iron deficiency. The chronic malarial infection may serve as an explanation; however, iron deficiency was not ruled out. Additional studies are necessary to determine the precise nature and cause of the erythrocytic microcytosis seen here.

Further studies are necessary to evaluate other potential physiologic and pathologic alterations caused by malarial infection which may have an impact on biomedical research. In particular, attention has been given to immunologic alterations caused by malaria in experimental and natural infections of man and nonhuman primates (7,8).

[6]Aralen®. Winthrop Laboratories, Division of Sterling Drug, New York, NY.
[7]Vetalar®. Parke-Davis, Detroit, MI.
[8]Vacutainer®. Becton, Dickinson, and Co, Rutherford, NJ.
[9]Coulter Electronics, Hialeah, FL.

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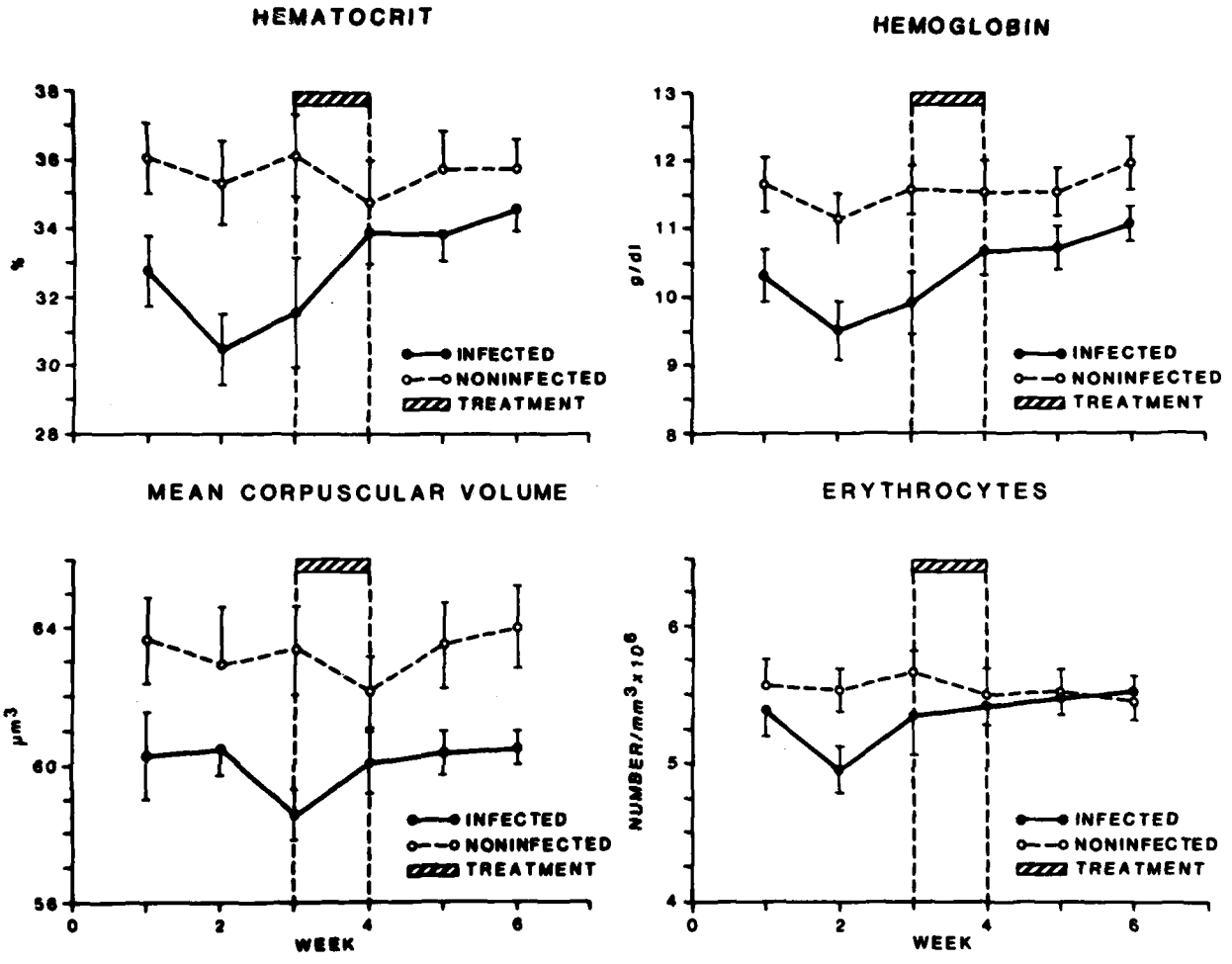

Figure 1
Hematologic values, pre- and post-treatment of 14 noninfected and 14 infected cynomolgus monkeys.

Table 1

Comparison of hematologic values in pre-treated versus post-treated and infected versus noninfected cynomolgus monkeys

	Pre-treatment versus post-treatment			Infected versus noninfected	
	4	5 (weeks)	6	Pre-treatment	Post-treatment
Hematocrit (%)	0.00[a]	0.05	0.01	0.01	NS[b]
Hemoglobin (g/dl)	0.05	0.01	0.001	0.01	NS
Erythrocytes (number/mm^3 × 10^6)	NS	0.05	0.01	NS	NS
Mean corpuscular volume (μm^3)	NS	NS	NS	0.05	0.05

[a]Probability ($p <$) value
[b]Not significant

A practical lesson regarding diagnosis of malaria was learned during this investigation. The parasitemia observed in many cases was extremely low with this subclinical manifestation in the cynomolgus monkey, with perhaps daily fluctuation caused by the periodicity of the organism. Because of these reasons, a highly accurate accounting of positively infected animals can only be obtained by examining blood on at least three consecutive days. Our results revealed a higher incidence of infection by this method than any single daily determination would have shown.

When treating nonhuman primate malaria, drug selection must be predicated on accurate species identification of the organism in order to insure efficacy. This is particularly true of the cynomolgus monkey in which the various malarias may or may not have a persistent tissue phase. The potential for mixed infections further complicate drug selection. In this study, only pure infections of *Plasmodium inui* were observed. Chloroquine, a drug effective in treating nonrelapsing malarias, was successful in clearing parasitemias. *Plasmodium inui* is a quartan-type malaria without a persistent tissue phase and thus was expected to be susceptible to chloroquine treatment.

Based on these results, we recommend that all cynomolgus monkeys from malaria endemic areas be

screened for malaria before use in biomedical research. Species identification of the malaria parasite and appropriate treatment should be instituted and follow-up blood smears examined to insure efficacy of treatment. In some instances, it may be advisable to presumptively treat all imported cynomolgus monkeys for both relapsing and nonrelapsing malaria in order to insure elimination of parasites from animals with nonpatent infections. Investigators contemplating the use of wild-caught cynomolgus monkeys should be made aware of the occurrence and consequences of this disease. If treatment is instituted prior to use, investigators should also be informed of the antimalarial drug utilized and its potential adverse side-effects.

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